CERTIFICATE OF MAILING

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST-CLASS MAIL IN AN ENVELOPE ADDRESSED TO: ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231, ON SEPTEMBER 24, 1999

Marianne H Michel
AGENT/ATTORNEY FOR APPLICANT

September 24, 1999
DATE

Attorney Docket No. 0448D

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Zhao *et al.*	Date:	September 24, 1999
Serial No.:	08/963,096	Group Art Unit:	1649
Filed	November 3, 1997	Examiner:	G. Benzion
For:	"METHODS FOR AGROBACTERIUM MEDIATED TRANSFORMATION OF PLANTS"		

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF WILLIAM J. GORDON-KAMM UNDER 37 CFR § 1.132

I, William J. Gordon-Kamm, declare:

I am a citizen of the United States of America and a resident of Urbandale, Iowa, United States of America.

I received a degree of Doctor of Philosophy in the area of Plant Physiology from Cornell University, Ithaca, New York, October 1984.

I received a degree of Master of Science in Botany from Western Washington University, Bellingham, Washington December 1981.

I received a degree of Bachelor of Science in Botany from Western Washington University, Bellingham, Washington December 1978.

I presently hold the position of Research Manager, Maize Elite Transformation at Pioneer Hi-Bred International, Inc., Johnston, Iowa, June 1996 to present.

I was employed as Coordinator, Maize Elite Transformation at Pioneer Hi-Bred International, Inc., Johnston, Iowa, and November 1994 to June 1996.

I was employed as Senior Research Scientist at DeKalb Plant Genetics, Mystic, Connecticut, February 1991 to October 1994. My work focused on improving drought tolerance in maize through transgenic approaches.

I was employed as a Research Scientist at DeKalb Plant Genetics, Mystic, Connecticut, May 1987 to February 1991. My work focused on the development and improvement of maize transformation methods focusing first on A188xB73 derivatives and later on DeKalb proprietary inbreds.

I was employed as assistant professor at New Mexico Highlands University, Division of Science and Mathematics, Las Vegas, New Mexico, August 1985 to May 1987.

I was employed as visiting scientist by USDA/ARS, Cereal Rust Laboratory, University of Minnesota, St. Paul, Minnesota, May to August 1986. My work focused on developing monocot transformation methods.

I was employed as preferred post-doctoral candidate by USDA/ARS, Cereal Rust Laboratory, University of Minnesota, St. Paul, Minnesota, October 1984 to September 1985. My work focused on developing microinjection methods for host-pathogen cell biology studies.

I am an inventor on the following patents:

US5550318. Methods and composition for the production of stably transformed, fertile monocot plants and cells thereof. DeKalb Genetics Corp. (see also; EP0485506 & WO9102071)

US5489520. Process of producing fertile transgenic *Zea mays* plants and progeny comprising a gene encoding phosphinothricin acetyl transferase. DeKalb Genetics Corp.

US5874265. Methods and composition for the production of stably transformed fertile monocot plants and cells thereof. DeKalb Genetics Corp. (see also; EP0721509 & WO95506128)

US5736369. Method for producing transgenic cereal plants (meristem transformation). Pioneer Hi-Bred International, Inc. (see also; EP0772687 & WO 9604392)

US5780709. Transgenic maize with increased mannitol content. DeKalb Genetics Corp. (see also; EP0889967 & WO 9726365)

US5929301. Nucleic acid sequence encoding FLP recombinase (use of maize-optimized FLP to excise sequences in plants). Pioneer Hi-Bred International, Inc.

I am an author on the following journal articles:

Laurie, J.D., G. Zhang, L.E. MCGann, W.J. Gordon-Kamm and D.D Cass. 1999. A novel technique for the isolation of embryo sacs from maize and the subsequent regeneration of plants. In Vitro Cellular & Developmental Biology (in press).

Sun, Y., B.P. Dilkes, C. Zhang, R.A. Dante, N.P. Carneiro, K.L. Lowe, R. Jung, W.J. Gordon-Kamm and B.A. Larkins. 1999. Characterization of maize (Zea mays L.) Wee1 and its activity in developing endosperm. Proc. Natl. Acad. Sci USA 96:4180-4185.

Lowe, K., Bowen, B., Hoerster, G., Ross, M., Bond, D., Pierce, D. and B. Gordon-Kamm. 1995. Germline Transformation of maize following manipulation of chimeric shoot meristems. Bio/Technology 13:677-682.

I am personally familiar with the method of collection of the data provided in Exhibits 1A and 2A and feel qualified to evaluate the results and form conclusions.

Single (or low) copy numbers of an integrated transgene is the preferred outcome of maize transformation experiments aimed at product development. It is well established in the literature that multicopy transgene integration is often accompanied by a high proportion of transgene rearrangement [Register et al., 1994. Plant Mol. Biol. 25:951-961]. In addition, other studies have shown that multiple gene copies can

correlate with transgene silencing or co-suppression, also referred to as trans-inactivation [Jorgenesen, R. 1991. AgBiotech News Inf 4: 265N-273N; Hobbs et al., 1993. Plant Mol. Biol. 21: 17-26; Jorgensen, R. 1993. Phil Trans. R. Soc. London B 339:173-181].

Our experimentation has clearly shown substantial differences between transgenic maize plants produced through particle-mediated DNA delivery (i.e. particle bombardment) compared to *Agrobacterium*-mediated delivery.

Side-by-side transformation experiments were performed in the same germplasm (tested for both Hi-II and a proprietary PHI inbred, designated N1) and the transgenic plants were evaluated for transgene copy number and expression. Southern analysis was performed to evaluate transgene copy number. A "desirable" transgenic event was defined as having a low copy number (1-3), and thus "non-desirable" transgenic events contained more than 3 transgene copies. Approximately 90-100 transgenic events generated by each method were analyzed for each genotype. *Agrobacterium*-mediated transformation was performed essentially as described in the present application. Particle bombardment transformation was performed essentially as described in US 5,484,596. For Hi-II, *Agrobacterium*-mediated transformation produced desirable (low copy) events at a 58% frequency, Southern Blot Exhibit 1A, while for particle bombardment the incidence of desirable events dropped to 8% frequency, Southern Blot Exhibit 2A. For both Southerns the DNA was digested with enzymes that drop out the intact expression cassette. For the inbred N1, the frequency of desirable *Agrobacterium*-generated events was 67%, while for particle bombardment only 13% of the events were desirable (low copy).

Transgene expression was also greatly enhanced in *Agrobacterium*-generated transgenic events relative to particle bombardment. For these experiments, a Bt-toxin expressing gene, cry1-f, driven by the maize ubiquitin promoter was introduced into maize using particle-mediated DNA delivery and *Agrobacterium*-mediated delivery transformation methods. For Hi-II transformants generated using *Agrobacterium*,

approximately 1350 transgenic plants were analyzed for cry1-f expression (using ELISA analysis to quantitate the level of transgene-encoded Bt-protein extracted from the plant tissue). The average expression level was 1889 ppm. For bombardment-generated transformants, plants from 20 events were analyzed and the average Bt ELISA results was 180 ppm. Later bombardment experiments generated an additional 72 events, that when analyzed gave a higher average ELISA value of 947 ppm. Experiments with Pioneer inbreds have born out this result; *Agrobacterium*-mediated transformation has consistently produced events with very high average expression levels (for example, inbred N1 produced an average ELISA value of 2666 pmm Bt-protein), and leaf-feeding bioassays have indicated very high levels of resistance to insects.

Based on the above results, and our cumulative experience since these initial experiments were performed, it's clear that *Agrobacterium*-mediated transformation of maize using the improved methods developed at Pioneer produced consistently better-quality transformants to advance for product development based on these two important criteria. *Agrobacterium*-mediated transformation produces transformants that more consistently exhibit 1) simple, stable, low-copy transgene integration, and 2) high transgene expression levels.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

William J. Gordon-Kamm

Dated: 9/24/99

EXHIBIT 1A

0.5 X 10⁹

Copies
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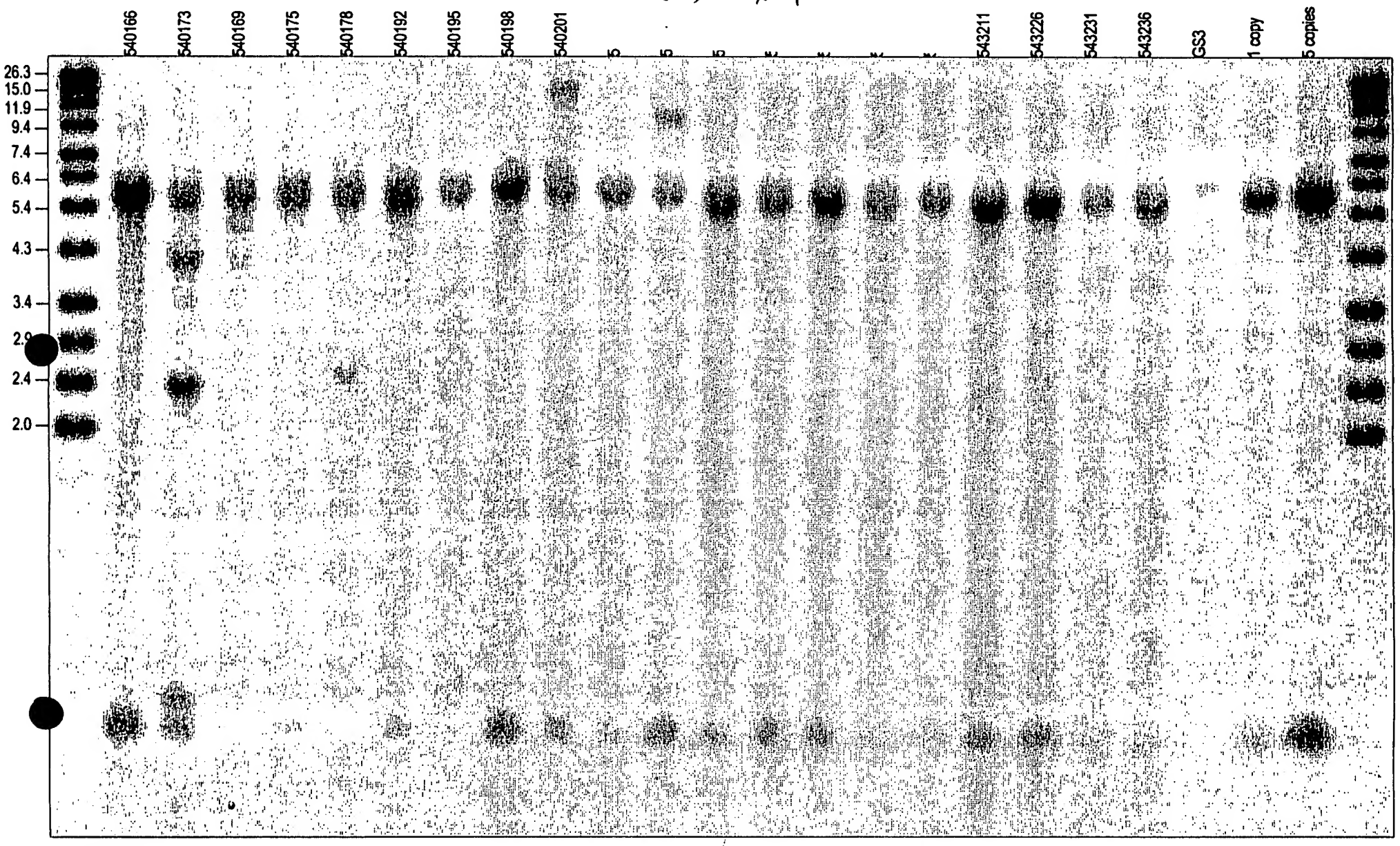

DIGEST: SphI
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EXPOSE: 24hrs
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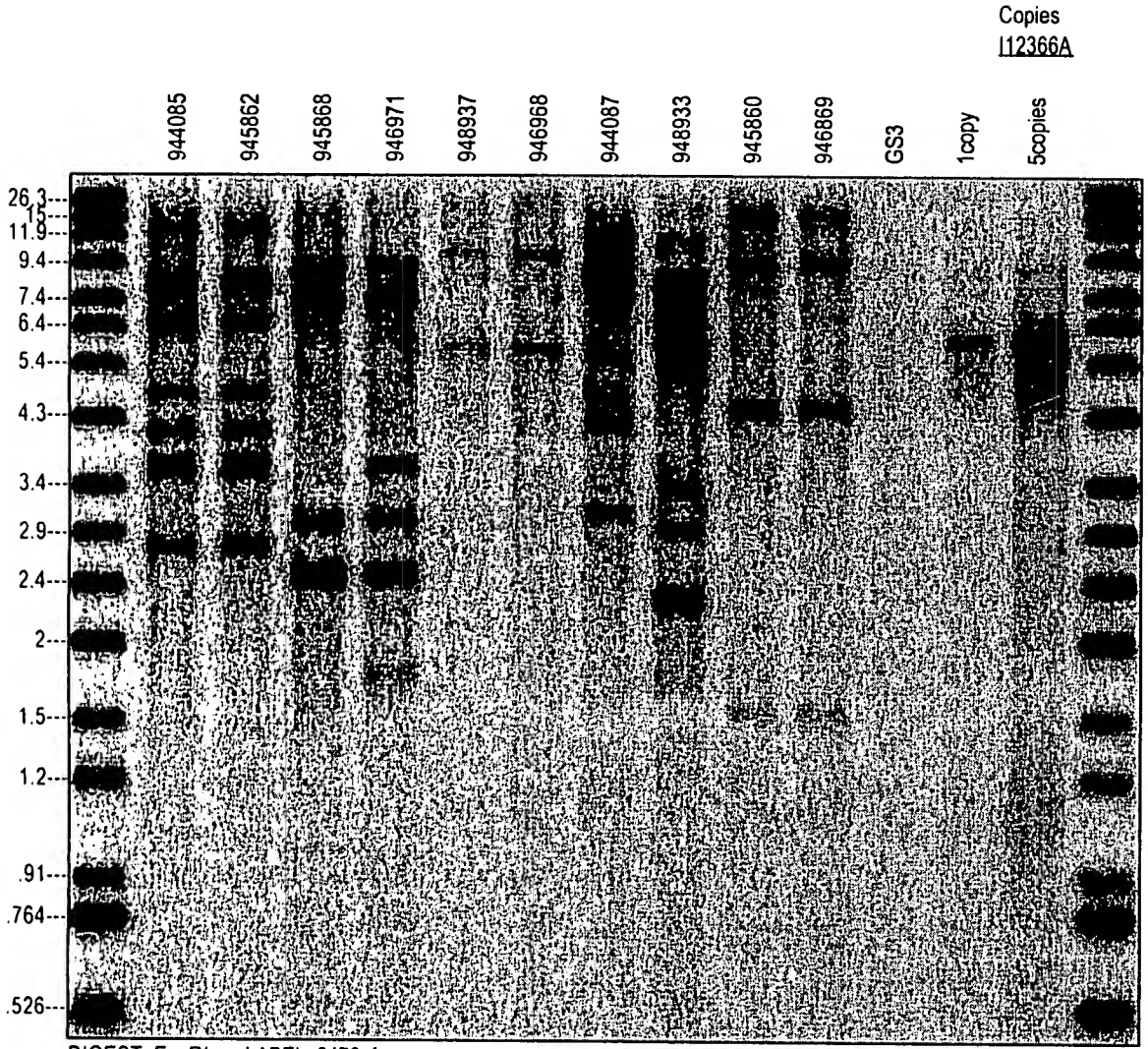
DATE: 10/29/96

LABEL: 60805aqb

EXHIBIT 2A



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DATE: 8/19/99